

Patent Claims

1. A method for the detection of cytosine methylations in DNA is hereby characterized in that

- a) the DNA to be investigated is brought into contact with a cytidine deaminase, whereby the cytidine deaminase deaminates cytidine and 5-methylcytidine at different rates,
- b) the partially deaminated DNA is investigated with respect to its sequence, and
- c) from the presence or the proportion of deaminated positions, conclusions can be made on the methylation status of the DNA to be investigated in said positions.

2. The method according to claim 1, further characterized in that *activation-induced cytidine deaminase – AID* or a biologically active fragment of AID or a modification thereof can be used as the methylation-specific cytidine deaminase.

3. The method according to one of claims 1 or 2, further characterized in that the DNA to be investigated is present at least partially in single-stranded form.

4. The method according to at least one of claims 1 to 3, further characterized in that the DNA to be investigated hybridizes with oligomers, whereby the hybrids are present in single-stranded form at the cytosine positions under investigation.

5. The method according to claim 4, further characterized in that the single-stranded regions are between 3 and 20 nucleotides long.
6. The method according to one of claims 4 or 5, further characterized in that the single-stranded regions are between 5 and 12 nucleotides long.
7. The method according to at least one of claims 4 to 6, further characterized in that the single-stranded region is 9 nucleotides long.
8. The method according to at least one of claims 1 to 7, further characterized in that the oligomers have a length of 20 to 150 nucleotides.
9. The method according to at least one of claims 1 to 8, further characterized in that the oligomers have a length of 35 to 60 nucleotides.
10. The method according to at least one of claims 4 to 9, further characterized in that the oligomers are present in a concentration of 1 pM to 1000 nM.
11. The method according to at least one of claims 4 to 10, further characterized in that the oligomers are present in a concentration of 1 nM to 100 nM.
12. The method according to at least one of claims 1 to 11, further characterized in that the DNA to be investigated is amplified after the enzyme treatment.

13. The method according to claim 12, further characterized in that the amplification is conducted by means of a polymerase reaction.

14. The method according to claim 13, further characterized in that the amplification is conducted by means of a polymerase chain reaction.

15. The method according to claim 14, further characterized in that the polymerase chain reaction is conducted by means of methylation-specific primers.

16. The method according to one of claims 14 or 15, further characterized in that at least one methylation-specific blocker oligomer is utilized in the polymerase chain reaction.

17. The method according to at least one of claims 12 to 16, further characterized in that a repeated enzymatic conversion with a cytidine deaminase is conducted after the amplification.

18. The method according to at least one of claims 12 to 17, further characterized in that the amplicates are analyzed by means of methods of length measurement, mass spectrometry or sequencing.

19. The method according to at least one of claims 12 to 17, further characterized in that the amplicates are analyzed by means of the primer extension method.

20. The method according to at least one of claims 12 to 17, further characterized in that the amplicates are analyzed by hybridization to oligomer arrays.

21. The method according to at least one of claims 12 to 17, further characterized in that the amplicates are analyzed with the use of real-time variants.

22. The method according to claim 21, further characterized in that a Taqman or a Lightcycler method is conducted.

23. The method according to at least one of claims 12 to 22, further characterized in that several fragments are simultaneously amplified by means of a multiplex reaction.

24. Use of a method according to at least one of claims 1-23 for the diagnosis of cancer diseases or other disorders associated with a change in the methylation status.

25. Use of a method according to at least one of claims 1 to 23 for predicting undesired drug interactions, for the differentiation of cell types and tissues or for the investigation of cell differentiation.

26. Use of cytidine deaminases, which convert cytidine and 5-methylcytidine at different rates, for methylation analysis.

27. Use of cytidine deaminases, which convert cytidine and 5-methylcytidine at different rates, for the diagnosis of cancer diseases or other disorders associated with a change in the methylation status.

28. Use of cytidine deaminases, which convert cytidine and 5-methylcytidine at different rates, for predicting undesired drug interactions, for the differentiation of cell types and tissues or for the investigation of cell differentiation.

29. Use according to at least one of claims 24 to 28, further characterized in that the cytidine deaminase involves activation-induced cytidine deaminase (AID), a biologically active fragment of AID or a modification thereof.

30. A kit , which comprises the AID enzyme, a biologically active fragment of AID or a modification thereof as well as oligomers and the buffers necessary for the deamination, as well as optionally also a polymerase, primers and probes for an amplification and detection.